PATENT
Docket No.: 176/61442 (1196)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Miller et al.	)	Examiner: Sarae Bausch
Serial No.	:	10/541,044	)	Art Unit: 1634
Cnfrm. No.	:	1984	)	
Filed	:	January 1, 2004	)	
For	:	HYBRIDIZATION-BASED BIOSENSOR CONTAINING HAIRPIN PROBES AND USE THEREOF	)	

DECLARATION OF BENJAMIN L. MILLER UNDER 37 CFR §1.132

I, BENJAMIN L. MILLER, pursuant 37 C.F.R. §1.132, declare as follows:

1. I am an inventor of the above-identified application.

2. I am currently an Associate Professor in the Department of Dermatology at the University of Rochester, Rochester, NY. I have served in that capacity since 2002. From 1996 to 2002, I was an Assistant Professor in the Department of Chemistry at the University of Rochester.

3. I received a Ph.D. in Organic Chemistry in 1994 from Stanford University at Stanford, CA. I received a B.S. in Chemistry in 1988 from Miami University at Oxford, OH. My research interests include: study of the structure, function, and molecular interactions of biomolecules through the design, synthesis, and structural analysis of novel small-molecule ligands; and the design and assembly of detection devices that can be used for screening molecular interactions, including biosensors of the present invention.

4. I have reviewed the Office Action dated June 24, 2009, and the references cited therein. I am presenting this declaration to demonstrate that using the assembly techniques described in Herne et al., "Characterization of DNA Probes Immobilized on Gold Surfaces," *J Am Chem Soc* 119:8916-8920 (1997) ("Herne") with hairpin probes, as described in U.S. Patent No. 6,312,906 to Cass et al. ("Cass"), would not achieve a functional sensor chip.

5. Sensor chips were prepared by using a thiolated hairpin probe (5'-ThioC6-GGC ATC GCA CAT CTG GCA ATG AAC TAC CTC GAT AGC C-TAMRA-3').

The structure of this probe is shown in Exhibit A. Following the protocol and the optimal conditions described in Table 1 of Herne, the substrate was immersed in a 1 μM hairpin DNA probe solution in 1.0 M KH_2PO_4 for 120 minutes, rinsed with water, and subsequently immersed in 1.0 mM aqueous solution of 6-mercaptohexanol (MCH) for 60 minutes. As a direct comparison, the inventive method was carried out using the same type of substrate, which was soaked in a mixture solution containing 3 μM 3-mercapto-1-propanol (MP) and 300 nM hairpin DNA probe, i.e., at a 10:1 ratio, for 120 minutes. The sensor chips obtained from both methods were incubated overnight with a target solution containing the target molecule (5'-GGC TAT CGA GGT AGT TCA TTG CCA GAT GTG CGA TGC C-3') at a concentration of 2.5 μM. The fluorescence intensity was measured as described in Example 3 of the present application. The experiment was performed in duplicate.

6. As shown in Exhibit B, the Herne method leads to a sensor chip with reduced post-hybridization fluorescence intensity. Average post-hybridization intensity (13467 a.u.) is less than the pre-hybridization intensity (16240.5 a.u.). On the contrary, the average post hybridization intensity for the claimed sensor chip (Miller method) is approximately 20 times more than the pre-hybridization intensity. Thus, the Herne method fails to produce a functional sensor chip, let alone a chip that can achieve at least a 5-fold increase in fluorescent intensity when the chip is exposed to the target.

7. From the foregoing, the method of sensor fabrication described in Herne is incompatible with a hairpin probe. Using the fabrication processes of Herne with hairpin probes from Cass would lead to a sensor chip that is non-functional and unusable. Therefore, one of skill in the art would not have expected that the combination of Cass and Herne would achieve a sensor chip of the presently claimed subject matter.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: NOVEMBER 23, 2009

Benjamin L. Miller

Exhibit A: Structure and Sequence of Hairpin Probe

Exhibit A:

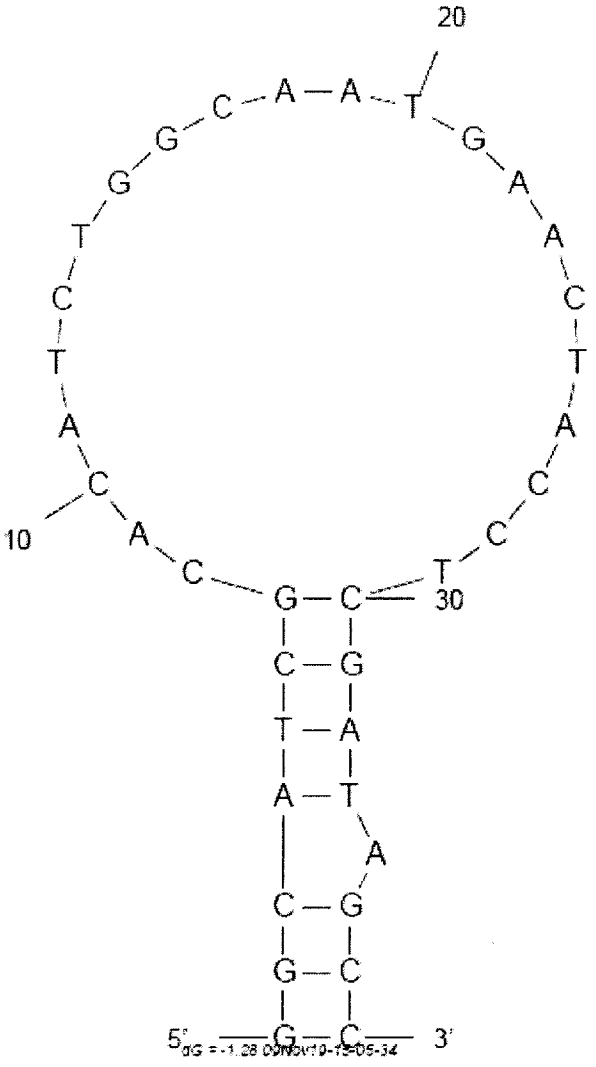

Exhibit B: Experimental Results

Exhibit B

Herne method

	Pre	Post	Gain
Chip1	15436	13252	-2184
	17068	15060	-2008
	15460	14184	-1276
	20292	15980	-4312
Chip2	16752	10676	-6076
	16652	12640	-4012
	16104	13820	-2284
	12160	12124	-36
Average	16240.5	13467	-2773.5
σ	2250.58	1682.47	-568.11

Gain = Post - Pre

[Probe]	1 μM
[Target]	2.5 μM
[MCH]	1 mM

Miller method

	Pre	Post	Gain
Chip1	639	18407	17768
	683	18669	17986
	584	18582	17998
	1162	18621	17459
Chip2	490	17743	17253
	1520	16153	14633
	1109	17296	16187
	780	17721	16941
	870.88	17899	17028.1
	356.07	870.704	514.634

[Probe]	300 nM
[Target]	2.5 μM
[MP]	3 μM

Target Incubation time: over night
CCD exposure time: 1 s
Probe: 5'-ThioC6- GGC ATC GCA CAT CTG GCA ATG AAC TAC CTC GAT AGC C- TAMRA-3' (disulfide)

Target: 5'-GGC TAT CGA GGT AGT TCA TTG CCA GAT GTG CGA TGC C-3'
MCH: 6-mercapto-1-hexanol
MP: 3-mercapto-1-propanol